Impact of nitrogen addition and precipitation on net ecosystem exchange in the Urat desert steppe, China

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Abstract: Amid global climate change, rising levels of nitrogen (N) deposition have attracted considerable attention for their potential effects on the carbon cycle of terrestrial ecosystems. The desert steppes are a crucial yet vulnerable ecosystem in arid areas, but their response to the combination of N addition and precipitation (a crucial factor in arid areas) remains underexplored. This study systematically explored the impact of N addition and precipitation on net ecosystem exchange (NEE) in a desert steppe in northern China. Specifically, we conducted a 2-a experiment from 2022 to 2023 with eight N addition treatments in the Urat desert steppe of Inner Mongolia Autonomous Region, China, to examine changes in NEE and explore its driving factors. The structural equation model (SEM) and multiple regression model were applied to determine the relationship of NEE with plant community characteristics and soil physical-chemical properties. Statistical results showed that N addition has no significant effect on NEE. However, it has a significant impact on the functional traits of desert steppe plant communities. SEM results further revealed that N addition has no significant effect on NEE in the desert steppe, whereas annual precipitation can influence NEE variations. The multiple regression model analysis indicated that plant functional traits play an important role in explaining the changes in NEE, accounting for 62.15% of the variation in NEE. In addition, plant height, as an important plant functional trait indicator, shows stronger reliability in predicting the changes in NEE and becomes a more promising predictor. These findings provide valuable insights into the complex ecological mechanisms governing plant community responses to precipitation and nutrient availability in the arid desert steppes, contributing to the improved monitoring and prediction of desert steppe ecosystem responses to global climate change.

Keywords: nitrogen addition; net ecosystem exchange; plant functional traits; structural equation model (SEM); multiple regression model; Urat desert steppe

1 Introduction

Nitrogen (N) addition plays a pivotal role in global climate change and has been closely linked to

the significant global warming observed since the Industrial Revolution (Chen et al., 2022). It is widely recognized for its profound influence on the structure and function of arid grassland ecosystems (Feng, 2022; Guo et al., 2022). Numerous studies have demonstrated that N addition can alter the structure and composition of plant communities (Chalcraft et al., 2008; Clark and David, 2008; Li et al., 2015), as well as their functional characteristics (Wan et al., 2008; Huang et al., 2012), ultimately affecting ecosystem functions (Baez et al., 2007; Liu et al., 2016b). In the grassland ecosystems, N is the primary limiting factor for plant growth, significantly affecting biosynthesis and photosynthesis (Duan et al., 2007; Zhao and Liu, 2009; Wang and Liu, 2014; Du et al., 2016; Zhai et al., 2017). N addition can alter this limitation on plant photosynthesis (Aber et al., 2003; Zhang et al., 2021b) and play a crucial role in regulating the activity of plant photosynthetic organs (Maroco et al., 2002). Furthermore, N addition may influence the distribution and efficiency of photosynthetic energy use (Zhang et al., 2009). For instance, N deficiency or excess can lead to changes in leaf physiology, including reductions in chlorophyll and related enzyme levels, as well as deceases in assimilative capacity and enzymatic activity (Wang and Xu, 2005). Increasing soil N content within a specific range can enhance leaf chlorophyll levels and photosynthetic enzyme activity, ultimately increasing the photosynthetic rate (Zhang et al., 2013a).

In the desert steppe, where water is a key driver of ecological processes, changes in precipitation can significantly affect the role of the ecosystem as a carbon dioxide (CO_2) source or sink (Reichstein et al., 2013; Wen et al., 2019). The desert steppe ecosystem is particularly sensitive to changes in precipitation (Zhang et al., 2014), which influences the ecosystem primarily through soil moisture changes (Chen et al., 2016). Excessive soil moisture can disrupt plant respiration and photosynthesis, potentially causing wilting or death (Pezeshki, 2001; Jackson and Ram, 2003). Conversely, soil moisture deficiency can cause water loss in plant leaves, reduce stomatal conductance, and damage photosynthetic components (Warren et al., 2004), ultimately lowering photosynthetic and transpiration rates (Lefi et al., 2004).

Net ecosystem exchange (NEE) is a critical metric for assessing carbon exchange dynamics between terrestrial ecosystems and the atmosphere. It reflects the net balance between CO_2 uptake through photosynthesis and release through respiration (Sloat et al., 2015; Wen et al., 2024). The impact of different ecosystems on NEE can vary widely due to differences in climate, soil conditions, plant communities, and N addition rates (Moinet et al., 2016; Tian et al., 2016). Although some studies have suggested that N addition can enhance net ecosystem carbon exchange (Xia et al., 2009; Zhang et al., 2021a), the differing responses of gross ecosystem productivity and ecosystem respiration to N addition across ecosystems have sparked debate on its overall impact (Niu et al., 2010). To better understand the impact of N addition on NEE, it is crucial to explore the combined effects of N deposition and precipitation changes, particularly in desert steppes, where these dynamics are likely to play a significant role.

The Urat desert steppe is a representative desert steppe within the temperate grassland region in northern China. It has been observed that N addition has a direct impact on plant functional traits and soil properties of the Urat desert steppe (He et al., 2021). Human-induced N deposition, coupled with changes in precipitation frequency and intensity, profoundly alters the structure and function of this desert steppe ecosystem. The Urat desert steppe is dominated by plant species such as *Stipa glareosa* P. A. Smirn., *Allium polyrhizum* Turcz. ex Regel, and *Peganum harmala* Linn. (Li et al., 2024), and changes in the photosynthetic characteristics of these plant species are expected to play a critical role in shaping the functions of this desert steppe ecosystem. To address the changes in the structure and function of the desert steppe as well as the photosynthetic characteristics of plant species, we conducted a 2-a experiment in the Urat desert steppe, employing eight different N addition treatments to examine the impact of N addition and precipitation on NEE and explore its driving factors. Specifically, we aimed to (1) assess the effects of N addition on plant community characteristics in the desert steppe; (2) determine the relationships between plant community characteristics and NEE under N addition treatments; and (3) examine the correlation between precipitation and NEE. These findings may highlight the complex ecological mechanisms driving the physiological responses of the plant community to

precipitation and nutrient dynamics in the arid desert steppes, offering new insights into monitoring and predicting ecosystem responses to climate change.

2   Study area and methods

2.1   Study area

The study was conducted at the herbaceous N addition experimental site of the Urat Desert-Grassland Research Station (41°25′N, 106°58′E; 1650 m a.s.l.; Fig. 1) in Bayannur City, Inner Mongolia Autonomous Region, China. The site is situated within desert steppe habitats. The region experiences a typical continental arid climate characterized by an average annual temperature of 5.30°C. The average annual precipitation is 155.6 mm, with approximately 80% occurring between April and August. Specifically, the annual average temperatures in 2022 and 2023 were 6.24°C and 6.75°C, respectively, while the annual precipitation amounts were 154.7 and 103.7 mm, respectively. The temperature peaked from June to August, and the precipitation reached its maximum during the same period (Fig. 2). The dominant soil type is gray-brown desert soil (Zhao et al., 2014; Wang et al., 2016). The vegetation type is predominantly desert steppe, dominated by *S. glareosa*, *A. polyrhizum*, *P. harmala*, and *Allium mongolicum* Regel.

2.2   Methods

2.2.1   Experimental design

The experimental plots were established in 2018 using a randomized block design with five replicates per treatment, resulting in 40 plots (8 treatments×5 replicates). Specifically, the experimental site was divided into five relatively uniform blocks; each further subdivided into eight 8.0 m×8.0 m plots where eight N addition treatments were randomly assigned. Buffer zones of 1.0 m were maintained between adjacent plots to prevent cross-contamination (Fig. 1). N was applied as urea in mid-to-late June and mid-to-late July. The eight N addition levels were 0.0 (N0; control), 0.5 (N0.5), 1.0 (N1), 3.0 (N3), 6.0 (N6), 12.0 (N12), 24.0 (N24), and 48.0 (N48) g/(m²·a). Among them, N0–N6 were considered as the low and middle N addition levels, while N12–N48 were regarded as the high N addition levels. Urea was dissolved in 10 L of water and evenly sprinkled onto each plot using a sprayer. For the N0 treatment, 10 L of clean water was sprayed in place of urea. Based on previous studies (Zong et al., 2019; Huang et al., 2021) and the environmental conditions of the Urat desert steppe, we infer that the N saturation threshold for herbaceous communities in this desert steppe is likely to be above 20.0 g/(m²·a).
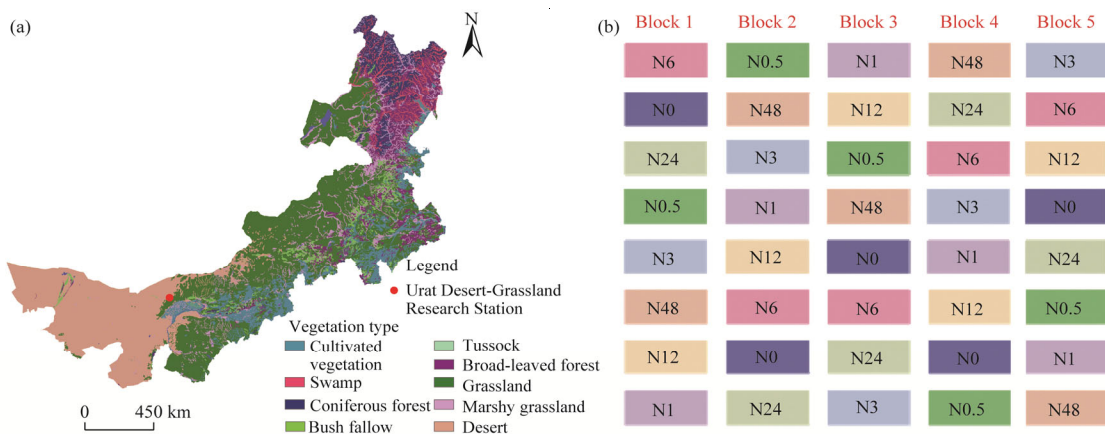

Fig. 1   Location of the study site (Urat Desert-Grassland Research Station) in Inner Mongolia Autonomous Region (a) and experimental design using a randomized block design (b). N0, N0.5, N1, N3, N6, N12, N24, and N48 represent the N addition levels of 0.0, 0.5, 1.0, 3.0, 6.0, 12.0, 24.0, and 48.0 g/(m²·a), respectively. Note that the figure is based on the standard map (GS(2020)4619) of the Ministry of Natural Resources of the People's Republic of China (https://www.mnr.gov.cn/sj/sjfw/), and the boundary of the standard map has not been modified.
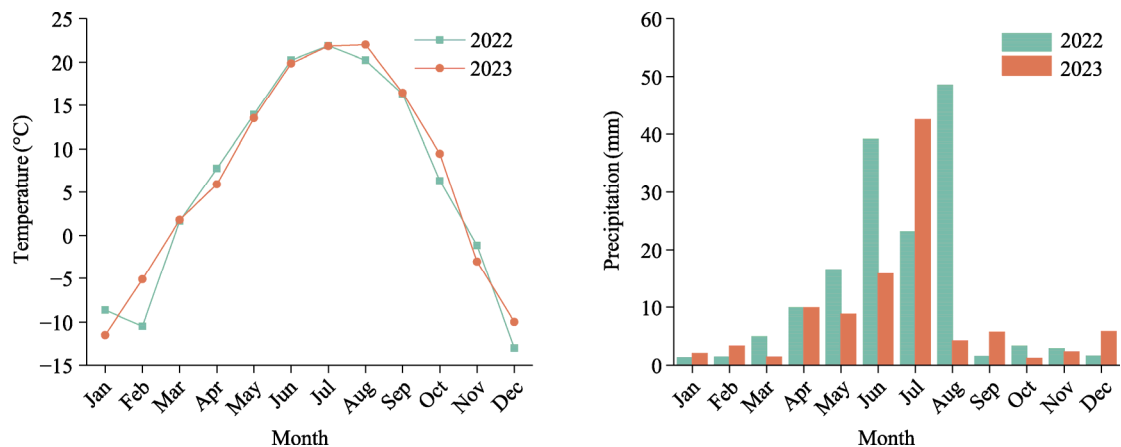
Fig. 2 Monthly average temperature (a) and monthly precipitation (b) at the Urat Desert-Grassland Research Station in 2022 and 2023

2.2.2 NEE

A custom-made assimilation chamber (0.5 m×0.5 m×0.5 m) was constructed using acrylic material with over 95% light transmittance. A fan was installed at the top and middle sections of the chamber to ensure even gas mixing and minimize shading effects. The chamber was connected to a LI-6400 Portable Photosynthetic System (LI-COR, Lincoln, Nebraska, USA), and a sealing strip was applied to the frame edges to ensure proper sealing during the measurements. The base of the assimilation chamber was a stainless-steel plate (0.5 m×0.5 m) inserted vertically into the ground by about 5.0 cm to maintain a level base. NEE measurements were taken during the peak growing season, from mid-July to early August in 2022 and 2023, on windless and sunny days between 09:00 and 11:00 (local standard time). In 2022, the NEE was measured thrice, while in 2023, it was measured twice. A fixed 0.5 m×0.5 m quadrat was randomly set in each plot, with the chamber placed directly over the quadrat. For each treatment, five replicates were performed, and each replicate was measured thrice. It should be note that NEE measurements of the N0.5 treatment for the first assessment in 2023 could not be conducted due to equipment failure.

2.2.3 Plant species composition and functional traits

In mid-August of 2022 and 2023, sampling was conducted in the central area of each experimental plot (8.0 m×8.0 m) to mitigate edge effects of the quadrats. In the central area of each experimental plot, leaves from the dominant plant species were collected. Approximately 20 fully expanded, healthy, and undamaged mature leaves were selected from each plant species. The leaves were then placed between filter papers moistened with deionized water, sealed in plastic bags, and transported to the laboratory at 4.00°C. In the laboratory, the leaves were immersed in deionized water at 4.00°C for 6 h. Subsequently, they were blotted dry with filter paper, and 10 leaves were randomly selected for scanning with Epson Perfection V330 Photo (Epson, Suwa, Japan). The leaf area was measured using WinRHIZO software (Regent Instruments, Sainte-Foy, Quebec, Canada), and leaf thickness (LT; mm) was determined using a vernier caliper (DL91150; Mitutoyo (China) Co., Ltd., Shanghai, China). Total saturated fresh weight was measured using an electronic balance with 0.0001 g precision. Finally, leaf dry weight was obtained by oven-drying at 65.00°C for 48 h until a constant weight was reached. Specific leaf area (SLA; m^2/kg) was calculated as the ratio of the single-sided area of a fresh leaf to its dry weight, while leaf dry matter content (LDMC; g/kg) was calculated as the ratio of the dry weight of a leaf to its saturated fresh weight.

Furthermore, approximately 2.0 g of mature leaves from the dominant plant species were collected, dried to a constant weight at 65.00°C for 48 h, ground, and sieved. The leaf carbon content (LCC; g/kg) and leaf N content (LNC; g/kg) were then determined using an elemental analyzer (Costech ECS 4010; NC Technologies, Milan, Italy). Additionally, a 1.0 m×1.0 m quadrat

was randomly selected within each experimental plot for vegetation surveys. Measurements included plant density (individuals/m^2), plant height (cm), and aboveground biomass (AGB; g/m^2) of dominant plant species. AGB was determined using the cutting method, followed by oven-drying at 65.00°C for 48 h to a constant weight (Liu et al., 2018). Finally, six plant functional traits (plant height, LT, SLA, LDMC, LNC, and LCC) were recorded, and the functional diversity of plant communities was calculated using FDiversity software (Casanoves et al., 2011).

Community-level functional diversity was quantified as the community-weighted mean (CWM) of the community traits using the following formula (Qiao et al., 2023):

$$CWM = \sum_{i=1}^{n} P_i \times trait_i, \tag{1}$$

where n is the number of species in the community; P_i is the relative biomass of species i in the community; and $trait_i$ is the trait value of species i.

2.2.4 Soil properties

One soil sample (100 cm^3) was collected from each plot using a ring knife, dried at 105.00°C for 48 h, and weighed to determine the soil bulk density (BD; g/cm^3). Furthermore, three soil samples were taken from the 0–10 cm soil layer in each plot using a soil drill with the diameter of 3.0 cm. These samples were mixed, air-dried in a well-ventilated area, and sieved using a 2-mm sieve to remove large stones for the measurements of total carbon content (TCC; g/kg), total nitrogen content (TNC; g/kg), pH, soil water content (SWC; %), and electrical conductivity (EC; μS/cm). Specifically, TCC and TNC were measured using a carbon and N analyzer (Costech Ecs4010, NC Technologies, Milan, Italy). Soil pH and EC were measured using an SX823 Portable PH Meter Conductivity Meter, while SWC was determined using the drying method described by Reynolds (1970).

2.3 Statistical analysis

A one-way analysis of variance (ANOVA) was conducted to evaluate the effects of N addition on plant community characteristics and NEE in the desert steppe. Data processing and analysis were performed using SPSS v.26.0 software (IBM Corp., Armonk, New York, USA), with treatment differences identified through Duncan's multiple range test. Structural equation model (SEM) was constructed using AMOS v.20.0 software (IBM Corp., Armonk, New York, USA) and the "lme4" package in R software (v.4.1.2, R Core Team, 2021). The relationship among NEE, plant community diversity, and soil properties was established using the "lm" function in R. Regression fitting was performed and then multiple regression analysis was carried out to investigate the effects of plant diversity and soil properties on NEE. Graphs and figures were generated using Origin v.2022 software.

3 Results

3.1 Effects of N addition on soil physical-chemical properties and plant functional traits

BD and TCC showed no significant changes across all N addition treatments (N0–N48) ($P>0.05$; Table 1). However, EC under the middle and high N addition levels increased significantly compared to the N0 treatment ($P<0.050$). SWC also increased significantly as increasing N addition level, while pH decreased significantly. Notably, SWC and TNC were significantly higher under high N addition levels (N12–N48) than under low and middle N addition levels (N0–N6) ($P<0.050$).

Different N addition treatments significantly influenced the CWM of plant height ($P<0.010$) and had highly significant effects on the CWM values of LT, SLA, LDMC, LNC, and LCC ($P<0.001$; Fig. 3). Specifically, compared with the N0 treatment, the CWM values of LT, SLA, and LNC increased significantly under the middle and high N addition levels (N6–N48) ($P<0.050$), whereas the CWM values of plant height, LDMC, and LCC decreased significantly under these treatments. Notably, the CWM value of SLA under the N24 treatment was significantly higher than those under other N addition treatments ($P<0.050$; Fig. 3a). The CWM

value of LDMC under the N24 treatment was significantly lower compared to other N addition treatments ($P<0.050$; Fig. 3b). The CWM values of LNC under the N6, N12, N24, and N48 treatments were significantly higher than those under other N addition treatments ($P<0.050$; Fig. 3c). Conversely, the CWM value of LCC under the N24 treatment was significantly lower than those under other N addition treatments ($P<0.050$; Fig. 3d). Additionally, LT exhibited a significantly higher CWM value under the N24 treatment than under other N addition treatments ($P<0.050$; Fig. 3f). In summary, the responses of the six CWM traits under the N24 treatment differed significantly from those under other N addition treatments.

Table 1   Changes in soil physical-chemical properties under different nitrogen (N) addition treatments

Treatment	BD (g/cm³)	EC (μS/cm)	pH	SWC (%)	TNC (g/kg)	TCC (g/kg)
N0	1.54±0.02[a]	44.06±2.31[c]	9.07±0.06[a]	1.38±0.17[b]	0.31±0.04[bc]	4.47±0.60[a]
N0.5	1.59±0.03[a]	43.82±3.39[c]	9.02±0.04[ab]	1.96±0.27[ab]	0.30±0.01[bc]	4.24±0.34[a]
N1	1.58±0.08[a]	41.34±1.75[c]	9.01±0.07[ab]	1.95±0.35[ab]	0.27±0.02[c]	4.03±0.37[a]
N3	1.65±0.06[a]	49.60±4.28[bc]	8.99±0.06[ab]	2.30±0.33[ab]	0.30±0.03[bc]	4.24±0.40[a]
N6	1.57±0.07[a]	51.36±4.18[bc]	8.93±0.05[ab]	1.55±0.36[ab]	0.29±0.04[bc]	4.07±0.66[a]
N12	1.70±0.09[a]	63.84±5.46[bc]	8.88±0.05[b]	2.88±0.81[a]	0.36±0.05[abc]	5.22±0.91[a]
N24	1.67±0.13[a]	75.84±11.21[b]	8.85±0.06[bc]	2.55±0.40[ab]	0.39±0.04[ab]	4.44±0.59[a]
N48	1.52±0.05[a]	110.56±18.83[a]	8.71±0.03[c]	2.22±0.33[ab]	0.43±0.04[a]	4.39±0.39[a]

Note: Mean±SE. BD, EC, SWC, TNC, and TCC refer to bulk density, electrical conductivity, soil water content, total nitrogen content, and total carbon content, respectively. N0, N0.5, N1, N3, N6, N12, N24, and N48 represent the N addition levels of 0.0, 0.5, 1.0, 3.0, 6.0, 12.0, 24.0, and 48.0 g/(m²·a), respectively. Different superscripted lowercase letters within the same column indicate significant differences between treatments at $P<0.050$ level.
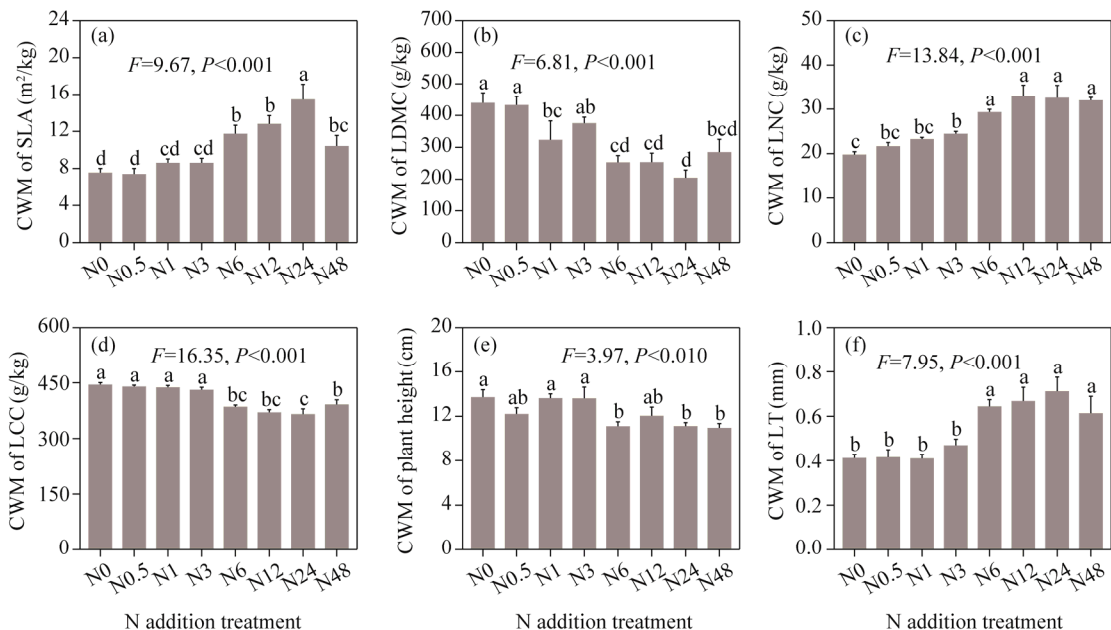

Fig. 3   Community-weighted mean (CWM) values of six functional traits under eight N addition treatments. (a), CWM of SLA (specific leaf area); (b), CWM of LDMC (leaf dry matter content); (c), CWM of LNC (leaf nitrogen content); (d), CWM of LCC (leaf carbon content); (e), CWM of plant height; (f), CWM of LT (leaf thickness). Different lowercase letters indicate significant differences in CWM values among different N addition treatments at $P<0.050$ level. Error bars indicate standard errors.

3.2   Effects of N addition on NEE

N addition had no statistically significant effect on NEE in the desert steppe ($P>0.050$; Fig. 4). In

2022, the first assessment showed that NEE values under the N24 and N48 treatments were lower than those under other N addition treatments, while N6 and N12 treatments showed significantly higher values of NEE ($P<0.050$; Fig. 4a). During the second assessment in 2022, NEE under the N1 treatment was significantly higher than those under other N addition treatments ($P<0.050$; Fig. 4b). The third assessment in 2022 showed that NEE under the N12 treatment consistently exceeded those under other N addition treatments (Fig. 4c). In 2023, the first assessment showed that NEE values under the N3 and N48 treatments were lower than those under other treatments (Fig. 4d). During the second assessment in 2023, the N24 treatment exhibited the lowest NEE value among all N addition treatments (Fig. 4e). A comparison between 2022 and 2023 showed that NEE values across all N addition treatments were higher in 2022 than in 2023 (Fig. 4f). Overall, N addition did not significantly affect NEE. N1–N12 treatments had a tendency to promote NEE, while N24 and N48 treatments had a slight inhibitory effect, but these effects were not statistically significant.
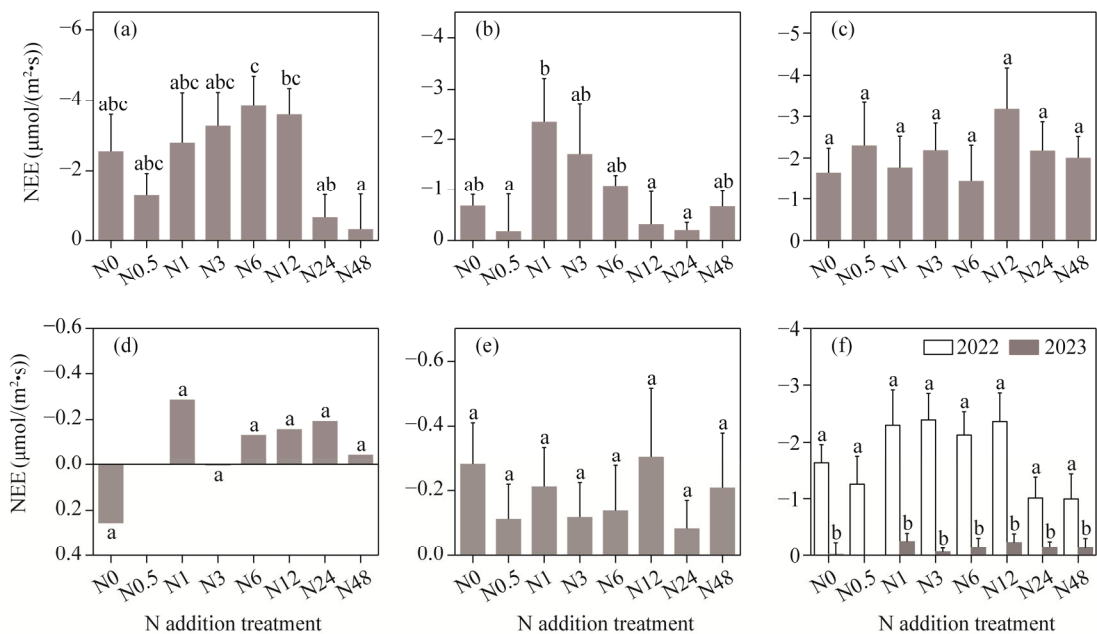

Fig. 4 Effects of N addition on net ecosystem exchange (NEE) under eight N addition treatments in 2022 and 2023. (a), the first assessment of NEE in 2022; (b), the second assessment of NEE in 2022; (c), the third assessment of NEE in 2022; (d), the first assessment of NEE in 2023; (e), the second assessment of NEE in 2023; (f), a comparison of the average NEE between 2022 and 2023. Different lowercase letters indicate significant differences in NEE values among different N addition treatments at $P<0.050$ level. Error bars indicate standard errors. Note that the NEE measurements of the N0.5 treatment for the first assessment in 2023 could not be conducted due to equipment failure.

In 2022 and 2023, N addition treatments significantly affected species richness and plant density ($P<0.050$; Fig. 5a and b) but had no significant effect on AGB ($P>0.050$; Fig. 5c). Under the high N addition levels (N24 and N48), species richness values were lower than those of low and middle N addition levels, while plant density under these two treatments (N24 and N48) increased compared to the N0 treatment. Additionally, AGB under each N addition treatment was significantly higher in 2022 than in 2023.

3.3 Relationship of NEE with plant community characteristics and soil physical-chemical properties

SEM results further showed that N addition had no significant effect on NEE, while annual precipitation had a direct impact on NEE (Fig. 6). N addition, annual precipitation, species richness, and plant height totally accounted for 54.00% of the total variation in NEE. Although N
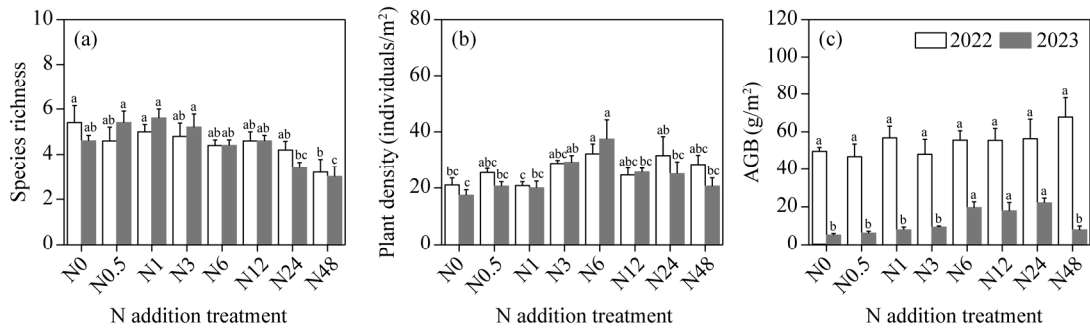
Fig. 5 Characteristics of plant communities in response to N addition treatments in 2022 and 2023. (a), species richness; (b), plant density; (c) AGB (aboveground biomass). Different lowercase letters within the same year indicate significant differences in plant community characteristics between N addition treatments at $P<0.050$ level. Error bars indicate the standard errors.

addition had a marginally significant direct and an indirect, nonsignificant effect on NEE (by reducing plant height), these positive effects were offset by an indirect, nonsignificant negative effect due to N-induced reduction in species richness. Consequently, N addition had no significant overall effect on NEE (Fig. 6).

The multiple regression model explained 37.00% of the variation in NEE (Fig. 7). Specifically, species diversity factors (species richness and plant density) accounted for 1.82% of the variation in NEE, plant functional traits (plant height, LT, SLA, LDMC, LCC, and LNC) accounted for 62.15%, and soil physical-chemical properties (TNC, TCC, pH, EC, SWC, and BD) accounted for 36.03%. As shown in the model, plant height significantly promoted NEE ($P<0.001$), while LDMC exerted a weaker effect on NEE ($P<0.100$).
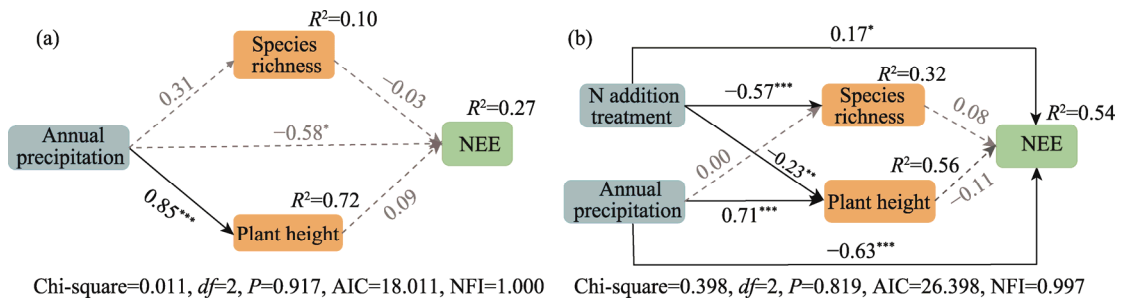

Fig. 6 Structural equation model (SEM) illustrating the relationship of NEE with annual precipitation, species richness, and plant height (a) and the relationship of NEE with N addition treatment, annual precipitation, species richness, and plant height (b). Single arrows indicate directional pathways in the model. The black solid line indicates significant relationship, while the gray dashed line indicates nonsignificant relationship. Standardized regression weights are displayed along the paths. The total variance is shown in the top-right corner of each rectangle, representing the influence of all predictors on that variable. Statistically significant pathways are denoted by the asterisks, with * representing $P<0.100$ level, ** representing $P<0.010$ level, and *** representing $P<0.001$ level. Chi-square, standardized Chi-square; df, degrees of freedom; AIC, Akaike Information Criterion; NFI, Normed Fit Index.

4 Discussion

N is a critical component of essential compounds, such as proteins, nucleic acids, and photosynthetic enzyme aliquots (Onoda et al., 2005; Ordoñez et al., 2009). It also serves as a primary factor driving photosynthesis in most desert steppe ecosystems. As a fundamental limiting element of grassland ecosystems, N plays a crucial role in regulating plant growth and development (Shi et al., 2024). The results of our study revealed that N addition had a significant effect on the functional traits of plant communities, indicating that N affects plant growth in the
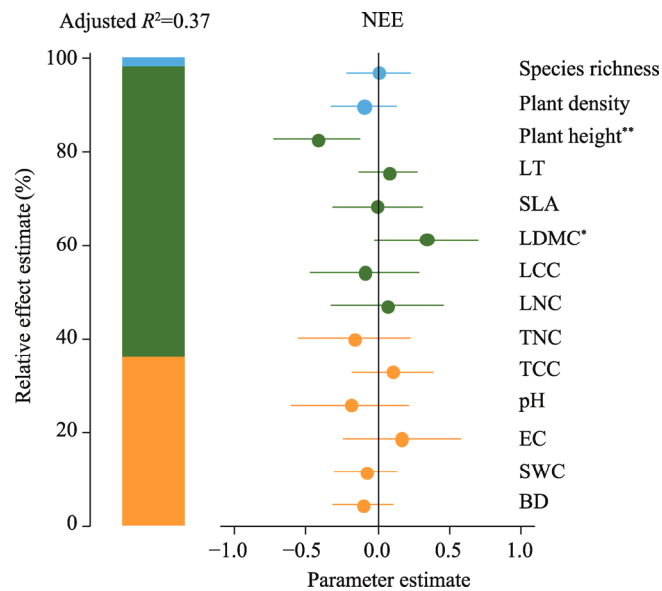
Fig. 7 Impact of multiple predictive factors on NEE. Each model includes the standardized regression coefficient (average parameter estimate) and its corresponding 95% confidence interval. Species diversity factors (in blue color) include species richness and plant density; plant functional trait factors (in green color) include plant height, LT, SLA, LDMC, LCC, and LNC; soil physical-chemical factors (in orange color) include TNC (total nitrogen content), TCC (total carbon content), pH, EC (electrical conductivity), SWC (soil water content), and BD (bulk density). The figure also shows the accounted percentage of variance for all factors, illustrating their relative importance in the model. [*], $P<0.100$ level; [**], $P<0.010$ level.

desert steppes. These findings align with numerous studies that have reported that exogenous N input promotes plant biomass accumulation in N-limited ecosystems (Bai et al., 2008; Wan et al., 2009). This effect is likely attributed to variations in plant ecological processes and scales involved in the assessed indicators.

Plant photosynthesis is affected by factors such as light, water, and CO_2 (Niu et al., 2003). In the Urat desert steppe region, relatively low precipitation during the growing season, coupled with high potential evaporation, makes water the primary environmental factor limiting plant growth (Cai et al., 2011). Understanding how changes in water availability affect photosynthetic characteristics is therefore crucial in the desert steppe ecosystems. NEE is a key parameter that reflects the photosynthetic capacity of plants (Feng, 2022). Our findings confirmed that precipitation is the main environmental factor influencing plant photosynthetic characteristics. This is because water increases the biomass of plant roots, enabling plant roots to absorb more water, support transpiration, and mitigate the water loss of plant leaves. This, in turn, improves the stomatal conductance of plant leaves, ultimately enhancing NEE (Ignace et al., 2007; Liu et al., 2013). However, our study revealed that N addition had no significant effect on NEE, contrasting with some earlier studies that have reported enhanced photosynthetic capacity of plants with appropriate N supplementation (Nakaji et al., 2001; An and Shangguan, 2008). Notably, our results support the findings of other studies that N addition has no significant impact on NEE (Xiao et al., 2010; Liu et al., 2016a). Furthermore, our results showed that plant functional traits accounted for 62.15% of the variation in NEE, with plant height emerging as a more reliable predictor. This indicates that the internal structure and functional traits of plant communities play an important role in regulating NEE. The complex interactions among plant species and their functional traits collectively regulate the carbon exchange process within ecosystems (Cheng et al., 2024). Variations in these processes can be attributed to differences in the plant species studied, the amount and timing of N addition, and the characteristics of the vegetation habitat. For example, water availability in the desert steppe ecosystems is a primary

factor limiting plant photosynthesis, and drought conditions may reduce the effectiveness of N addition (Wen et al., 2024). Additionally, N loss in the grassland ecosystems, caused by factors such as herbivore grazing and ammonia (NH_3) volatilization, further complicates the N cycle (Zhang et al., 2013b). The volatilization of NH_3 disrupts soil N balance, significantly limiting the ability of herbaceous plants to absorb and utilize N efficiently (Xu et al., 2022).

SEM results further confirmed that N addition did not significantly affect NEE, whereas annual precipitation directly affected it. These findings highlight the complex ecological mechanisms underlying the physiological responses of plant communities to precipitation and nutrient availability in the arid desert steppe ecosystems. Under simultaneous N addition, NEE in 2022 was significantly higher than that in 2023, primarily due to greater precipitation in 2022. When SWC reaches a certain threshold, it can stimulate the fertility effects of N, increasing chlorophyll stability and content, which in turn enhances the photosynthetic rate of plant leaves (Zhong et al., 2008). The results of this study confirmed that precipitation directly affects NEE, aligning with the findings of William Stanley (2007). Both water addition and N application have obvious effects on desert steppe ecosystems. Plant photosynthesis is influenced not only by intrinsic physiological factors but also by environmental conditions. Our study revealed that N addition had a higher effect on NEE in wet year (i.e., 2022) than in dry year (i.e., 2023), highlighting the severe challenge of water stress in the arid desert steppes, where annual precipitation is scarce. Studies in the alpine steppe ecosystems have shown that the total amount, distribution, and timing of precipitation collectively determine water availability for plant growth (Bai et al., 2020). Experiments simulating varying precipitation intensities on soil respiration and productivity on the Loess Plateau of China revealed that extreme precipitation affected vegetation net primary productivity, soil respiration, biodiversity, and soil physical-chemical properties such as SWC (Wang et al., 2018). These findings underscore the critical role of precipitation and SWC, consistent with the results of this study, which identified precipitation as a key factor influencing NEE. The observed change in NEE between years—higher in 2022 than in 2023—highlights the significant regulatory effect of natural interannual climate fluctuations on ecosystem functions, particularly changes in precipitation. This further emphasizes the pivotal role of water as a limiting factor in the arid ecosystems.

5   Conclusions

This 2-a experimental study conducted in the Urat desert steppe of Inner Mongolia explored the impact of N addition and precipitation on NEE. Contrary to expectations, N addition did not significantly affect NEE. However, it had a significant impact on the functional traits of desert steppe plant communities. Instead, the change of annual precipitation was the primary driver, directly influencing NEE, with plant functional traits accounting for much of the variation in NEE. Plant height was also identified as a key predictor for NEE.

While our findings contribute to understanding the effects of N addition on NEE in the arid desert steppe ecosystems under changing precipitation, this study has certain limitations. For instance, the 2-a experimental duration may not be sufficient to fully capture the long-term effects of N addition and precipitation changes on desert steppe ecosystems. Future studies with longer experimental durations are needed to further explore the complex ecological processes in this region. Nonetheless, our study underscores the complex ecological mechanisms in the arid desert steppes and offers valuable insights for understanding and predicting ecosystem responses to climate change.

Conflict of interest

Acknowledgements

Author contributions

Conceptualization: ZUO Xiaoan, ZHANG Rui, ZHANG Xiaoxue; Data curation: ZHANG Xiaoxue, WANG Zhengjiaoyi; Formal analysis: ZHANG Xiaoxue; Funding acquisition: ZUO Xiaoan; Investigation: ZHANG Xiaoxue, QIAO Jingjuan; Methodology: ZHANG Xiaoxue, YUE Ping; Project administration: ZUO Xiaoan; Resources: ZUO Xiaoan; Software: ZHANG Xiaoxue, SONG Zhaobin; Supervision: ZUO Xiaoan; Validation: ZHANG Xiaoxue; Visualization: ZHANG Xiaoxue; Writing - original draft preparation: ZHANG Xiaoxue; Writing - review and editing: ZUO Xiaoan, YUE Ping. All authors approved the manuscript.

References

Aber J D, Goodale C L, Ollinger S V, et al. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? BioScience, 53(4): 375–389.

An H, Shangguan Z P. 2008. Specific leaf area, leaf nitrogen content, and photosynthetic acclimation of *Trifolium repens* L. seedlings grown at different irradiances and nitrogen concentrations. Photosynthetica, 46(1): 143–147.

Baez S, Fargione J, Moore D I, et al. 2007. Atmospheric nitrogen deposition in the northern Chihuahuan Desert: temporal trends and potential consequences. Journal of Arid Environments, 68(4): 640–651.

Bai Y F, Wu J G, Xing Q, et al. 2008. Primary production and rain use efficiency across a precipitation gradient on the Mongolia Plateau. Ecology, 89(8): 2140–2153.

Bai Z, Li Y L, Shi C J, et al. 2020. Effects of different grazing seasons on plant communities in the vegetation green-up and peak growing seasons of a typical steppe. Chinese Journal of Grassland, 42(2): 67–75. (in Chinese)

Cai H X, Wu F Z, Yang W Q. 2011. Effects of drought stress on the photosynthesis of *Salix paraqplesia* and *Hippophae rhamnoides* seedlings. Journal of Ecology, 31(9): 2430–2436. (in Chinese)

Casanoves F, Pla L, Di Rienzo J A, et al. 2011. FDiversity: a software package for the integrated analysis of functional diversity. Methods in Ecology and Evolution, 2(3): 233–237.

Chalcraft D R, Cox S B, Clark C, et al. 2008. Scale-dependent responses of plant biodiversity to nitrogen enrichment. Ecology, 89(8): 2165–2171.

Chen M L, Zhang B W, Ren T T, et al. 2016. Responses of soil moisture to precipitation pattern change in semiarid grasslands in Nei Mongol, China. Journal of Plant Ecology, 40(7): 658–668. (in Chinese)

Chen X, Jiao T, Mu R, et al. 2022. Effects of nitrogen addition on growth and photosynthetic characteristics of *Elymus nutans* in alpine artificial grassland. Journal of Grasslands, 30(8): 1964–1971. (in Chinese)

Cheng S S, Xue W F, Gong X, et al. 2024. Reconciling plant and microbial ecological strategies to elucidate cover crop effects on soil carbon and nitrogen cycling. Journal of Ecology, 112(12): 2901–2916.

Clark C M, David T. 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature, 451: 712–715.

Du J, Shu S, Shao Q S, et al. 2016. Mitigative effects of spermidine on photosynthesis and carbon-nitrogen balance of cucumber seedlings under $Ca(NO_3)_2$ stress. Journal of Plant Research, 129(1): 79–91.

Duan Y H, Zhang Y L, Ye L T, et al. 2007. Responses of rice cultivars with different nitrogen use efficiency to partial nitrate nutrition. Annals of Botany, 99(6): 1153–1160.

Feng C X. 2022. Effects of N and water addition on photosynthetic characteristics of dominant plants in a typical temperate grassland community. MSc Thesis. Hohhot: Inner Mongolia University. (in Chinese)

Guo X X, Zuo X A, Yue P, et al. 2022. Direct and indirect effects of precipitation change and nutrients addition on desert steppe productivity in Inner Mongolia, northern China. Plant and Soil, 471: 527–540.

He K J, Huang Y M, Qi Y, et al. 2021. Effects of nitrogen addition on vegetation and soil and its linkages to plant diversity and productivity in a semi-arid steppe. Science of the Total Environment, 778: 146299, doi: 10.1016/j.scitotenv.2021.146299.

Huang J Y, Yu H L, Yuan Z Y, et al. 2012. Effects of long-term increased soil N on leaf traits of several species in typical Inner Mongolian grassland. Acta Ecologiga Sinica, 32(5): 1419–1427. (in Chinese)

Huang J Y, Xu Y X, Yu H L, et al. 2021. Soil prokaryotic community shows no response to 2 years of simulated nitrogen deposition in an arid ecosystem in northwestern China. Environmental Microbiology, 23(2): 1222–1237.

Ignace D D, Huxman T E, Weltzin J F, et al. 2007. Leaf gas exchange and water status responses of a native and non-native grass to precipitation across contrasting soil surfaces in the Sonoran Desert. Oecologia, 152(3): 401–413.

Jackson M B, Ram P C. 2003. Physiological and molecular basis of susceptibility and tolerance of rice plants to complete submergence. Annals of Botany, 91(2): 227–241.

Lefi E, Medrano H, Cifre J. 2004. Water uptake dynamics, photosynthesis and water use efficiency in field-grown *Medicago arborea* and *Medicago citrina* under prolonged Mediterranean drought conditions. Annals of Applied Biology, 144(3): 299–307.

Li W J, Liu H M, Zhao J N, et al. 2015. Effects of nitrogen and water addition on plant species diversity and biomass of common species in the *Stipa baicalensis* steppe, Inner Mongolia, China. Acta Ecologiga Sinica, 35(19): 6460–6469. (in Chinese)

Li X Y, Song Z B, Hu Y, et al. 2024. Drought intensity and post-drought precipitation determine vegetation recovery in a desert steppe in Inner Mongolia, China. Science of the Total Environment, 906: 167449, doi: 10.1016/j.scitotenv.2023.167449.

Liu H M, Li J, Chen X W, et al. 2016a. Photosynthetic characteristics of *Leymus chinensis* in response to simulated nitrogen deposition in Inner Mongolia, China. Ecology and Environmental Sciences, 25(6): 973–980. (in Chinese)

Liu H M, Li J, Huangfu C H, et al. 2016b. Photosynthetic characteristics and leaf functional properties of Baikal needlegrass in response to long-term nitrogen addition. Acta Prataculturae Sinica, 25(11): 76–85. (in Chinese)

Liu H Y, Mi Z R, Lin L, et al. 2018. Shifting plant species composition in response to climate change stabilizes grassland primary production. Proceedings of The National Academy of Sciences of The United States of America, 115(16): 4051–4056.

Liu Z B, Cheng R M, Xiao W F, et al. 2013. Effect of waterlogging on photosynthetic and physioecological characteristics of plants. Forestry Science, (3): 33–38. (in Chinese)

Maroco J P, Breia E, Faria T, et al. 2002. Effects of long-term exposure to elevated CO_2 and N fertilization on the development of photosynthetic capacity and biomass accumulation in *Quercus suber* L. Plant Cell and Environment, 25(1): 105–113.

Moinet G Y K, Cieraad E, Rogers G N D, et al. 2016. Addition of nitrogen fertiliser increases net ecosystem carbon dioxide uptake and the loss of soil organic carbon in grassland growing in mesocosms. Geoderma, 266: 75–83.

Nakaji T, Fukami M, Dokiya Y, et al. 2001. Effects of high nitrogen load on growth, photosynthesis and nutrient status of *Cryptomeria japonica* and *Pinus densiflora* seedlings. Trees, 15(8): 453–461.

Niu S L, Jiang G M, Li Y G, et al. 2003. Diurnal gas exchange and superior resources use efficiency of typical C_4 species in Hunshandak Sandland, China. Photosynthetica, 41: 221–226.

Niu S L, Wu M Y, Han Y, et al. 2010. Nitrogen effects on net ecosystem carbon exchange in a temperate steppe. Global Change Biology, 16(1): 144–155.

Onoda Y, Hikosaka K, Hirose T. 2005. Seasonal change in the balance between capacities of RuBP carboxylation and RuBP regeneration affects CO_2 response of photosynthesis in *Polygonum cuspidatum*. Journal of Experimental Botany, 56(412): 755–763.

Ordoñez J C, Van Bodegom P M, Witte J P M, et al. 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecology and Biogeography, 18(2): 137–149.

Pezeshki S R. 2001. Wetland plant responses to soil flooding. Environmental and Experimental Botany, 46(3): 299–312.

Qiao J J, Zuo X A, Yue P, et al. 2023. High nitrogen addition induces functional trait divergence of plant community in a temperate desert steppe. Plant and Soil, 487: 133–156.

R Core Team. 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reichstein M, Bahn M, Ciais P, et al. 2013. Climate extremes and the carbon cycle. Nature, 500: 287–295.

Reynolds S G. 1970. The gravimetric method of soil moisture determination Part I A study of equipment, and methodological problems. Journal of Hydrology, 11(3): 258–273.

Shi G X, Zhang Z H, Ma L, et al. 2024. Nitrogen addition drives changes in arbuscular mycorrhizal fungal richness through changes in plant species richness in revegetated alpine grassland. Fungal Ecology, 67: 101303, doi: 10.1016/j.funeco.2023.101303.

Sloat L L, Henderson A N, Lamanna C, et al. 2015. The effect of the foresummer drought on carbon exchange in subalpine meadows. Ecosystems, 18: 533–545.

Tian D S, Niu S L, Pan Q G, et al. 2016. Nonlinear responses of ecosystem carbon fluxes and water-use efficiency to nitrogen addition in Inner Mongolia grassland. Functional Ecology, 30(3): 490–499.

Wan H W, Yang Y, Bai S Q, et al. 2008. Variations in leaf functional traits of six species along a nitrogen addition gradient in *Leymus chinensis* steppe in Inner Mongolia. Journal of Plant Ecology, 32(3): 611–621. (in Chinese)

Wan S Q, Xia J Y, Liu W X, et al. 2009. Photosynthetic overcompensation under nocturnal warming enhances grassland carbon sequestration. Ecology, 90(10): 2700–2710.

Wang G L, Liu F. 2014. Carbon allocation of Chinese pine seedlings along a nitrogen addition gradient. Forest Ecology & Management, 334: 114–121.

Wang Q, Xu C Y. 2005. Affects of nitrogen and phosphorus on plant leaf photosynthesis and carbon partitioning. Shandong Forestry Science and Technology, (5): 59–62. (in Chinese)

Wang S K, Zhao X Y, Jia K F, et al. 2016. Soil bacterial diversity and its vertical distribution in *Stipa klemenzii* community of Urad desert Steppe. Journal of Desert Research, 36(6): 1564–1570. (in Chinese)

Wang Z P, Zhang X Z, He Y T, et al. 2018. Effects of precipitation changes on the precipitation use efficiency and aboveground productivity of alpine steppe-meadow on northern Tibetan Plateau, China. Journal of Applied Ecology, 29(6): 1822–1828. (in Chinese)

Warren C R, Livingston N J, Turpin D H. 2004. Photosynthetic responses and N allocation in Douglas–fir needles following a brief pulse of nutrients. Tree Physiology, 24(6): 601–608.

Wen C, Shan Y M, Xing T T, et al. 2024. Effects of nitrogen and water addition on ecosystem carbon fluxes in a heavily degraded desert steppe. Global Ecology and Conservation, 52: e02981, doi: 10.1016/j.gecco.2024.e02981.

Wen H Y, Wu S J, Fu H. 2019. Influence of nitrogen addition on net ecosystem carbon exchange of steppe in the Loess Plateau. Journal of Desert Research, 39(3): 34–40. (in Chinese)

William Stanley H, Daniel L P, Katharine N S. 2007. Ecosystem responses to water and nitrogen amendment in a California grassland. Global Change Biology, 13(11): 2341–2348.

Xia J Y, Niu S L, Wan S Q. 2009. Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. Global Change Biology, 15(6): 1544–1556.

Xiao S S, Dong Y S, Qi Y C, et al. 2010. Effects of mineral fertilizer addition on leaf functional traits and photosynthetic characteristics of *Leymus chinensis* from a temperate grassland in Inner Mongolia in China. Acta Scientiae Circumstantiae, 30(12): 2535–2543. (in Chinese)

Xu F L, Zhang J, Li Y, et al. 2022. Effects of fertilization methods on ammonia volatilization of spring maize in dry farming on the Loess Plateau. Scientia Agricultura Sinica, 55(12): 2360–2371. (in Chinese)

Zhang B, Zhu J J, Liu H M, et al. 2014. Effects of extreme rainfall and drought events on grassland ecosystems. Journal of Plant Ecology, 38(9): 1008–1018. (in Chinese)

Zhang H X, Li X R, Guan D X, et al. 2021a. Nitrogen nutrition addition mitigated drought stress by improving carbon exchange and reserves among two temperate trees. Agricultural and Forest Meteorology, 311: 108693, doi: 10.1016/j.agrformet.2021.108693.

Zhang R, Wang Y, Jin G Q, et al. 2013a. Nitrogen addition affects root growth, phosphorus and nitrogen efficiency of three provenances of *Schima superba* in barren soil. Acta Ecologica Sinica, 33(12): 3611–3621. (in Chinese)

Zhang X C, Shangguan Z P. 2009. The responses of photosynthetic electron transport and partition in the winter wheat leaves of different drought resistances to nitrogen levels. Plant Physiology Communications, 45(1): 13–18. (in Chinese)

Zhang X W, An H, Liu X P, et al. 2021b. Effects of short-term nitrogen addition on plant community composition and stability of desert steppe. Journal of Ecology, 40(8): 2400–2409. (in Chinese)

Zhang Y H, He N P, Zhang G M, et al. 2013b. Ammonia emissions from soil under sheep grazing in Inner Mongolian grasslands of China. Journal of Arid Land, 5(2): 155–165.

Zhao C Z, Liu Q. 2009. Growth and photosynthetic responses of two coniferous species to experimental warming and nitrogen fertilization. Canadian Journal of Forest Research, 39(1): 1–11.

Zhao X Y, Liu L X, Wang W, et al. 2014. Effects of precipitation fluctuations on productivity of desert grasslands. Journal of Desert Research, 34(6): 1486–1495. (in Chinese)

Zhong J D, Mo X Y, Liu J X. 2018. Growth response of *Praxelis clematidea* seedlings to water and nitrogen coupling. Pratacultural Science, 12(7): 1670–1678. (in Chinese)

Zong N, Zhao G S, Shi P L. 2019. Different sensitivity and threshold in response to nitrogen addition in four alpine grasslands along a precipitation transect on the Northern Tibetan Plateau. Ecology and Evolution, 9(17): 9782–9793.